Proc. Natl. Acad. Sci. USA
Vol. 78, No. 2, pp. 991–995, February 1981
Cell Biology

Ca^{2+}- and calmodulin-stimulated endogenous phosphorylation of neurotubulin

(phosphotubulin/tubulin phosphorylation/tubulin kinase/synaptic tubulin/brain calmodulin)

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Communicated by Dorothy M. Horstmann, October 8, 1980

ABSTRACT **Ca^{2+} plays a major role in the functional use of tubulin in brain and other tissues. It activates an endogenous tubulin kinase system in brain cytosol, tubulin, and presynaptic nerve terminal fractions prepared from rat brain. Activation of the Ca^{2+} tubulin kinase system was modulated by the Ca^{2+} receptor protein calmodulin. The concentrations of Ca^{2+} and calmodulin required to produce a half-maximal stimulation of the tubulin kinase were 0.8 μM and 0.4 μg, respectively. Ca^{2+}-calmodulin tubulin kinase activity was very unstable after death, and procedures were developed to stabilize the activity of this enzyme system. Evidence is presented demonstrating that the Ca^{2+}-calmodulin tubulin kinase system is distinct from the previously described cyclic AMP-Mg^{2+} tubulin kinase. The results suggest that Ca^{2+}- and calmodulin-stimulated phosphorylation of tubulin may be a major biochemical mechanism modulating some of calcium's effects on tubulin and may play a significant role in mediating some of calcium's actions on cell functions.**

Tubulin is found in high concentrations in brain (1) and has been implicated in several physiological processes associated with nerve cell function (2–4). Ca^{2+} has been shown to play a major role in the functional use of tubulin (5–7). Given the significance of Ca^{2+} in the function of the nervous system (8, 9), it is important to understand the biochemical effects of Ca^{2+} on tubulin. However, little is known about the molecular mechanism of calcium's action on tubulin or the extent to which calcium's effects on tubulin might mediate the actions of Ca^{2+} on cell function.

Previous investigations in this laboratory demonstrated that Ca^{2+} stimulates endogenous protein phosphorylation in several brain fractions (10–12). Although Ca^{2+} was shown to stimulate the phosphorylation of many brain proteins, two phosphoproteins with molecular weights similar to those of α- and β-tubulin were consistently major substrates for Ca^{2+}-dependent protein kinases, and the effect of Ca^{2+} on the phosphorylation of these proteins was shown to be dependent on the Ca^{2+} receptor protein calmodulin (13, 14). These observations stimulated our interest in studying the possible effects of Ca^{2+} and calmodulin on the endogenous phosphorylation of rat brain tubulin.

This investigation demonstrates the existence in brain of a Ca^{2+}- and calmodulin-stimulated tubulin kinase system that is distinct from the previously described cyclic AMP-stimulated tubulin kinase (15, 16). The Ca^{2+} tubulin kinase system is also present in preparations of presynaptic nerve terminals. The possible roles of Ca^{2+}-calmodulin tubulin kinase in mediating some of the effects of Ca^{2+} on tubulin and nerve cell functions are discussed. A preliminary note about this research has been published (17).

MATERIALS AND METHODS

[γ-^{32}P]ATP (5–10 Ci/mmol; 1 Ci = 3.7×10^{10} Bq) as the triethylammonium salt was obtained from New England Nuclear. *Staphylococcus aureus* protease (Miles), papain (Sigma), phenylmethylsulfonyl fluoride (PMSF; Sigma), and ampholytes [pH 5–7, 40% (wt/vol), and pH 3–10, 40% (wt/vol)] (Bio-Rad) were used as supplied.

Brain cytosol was prepared as follows. Sprague–Dawley female rats (100–150 g) were decapitated, and cortexes were dissected free and immediately homogenized (12), 1:1 (wt/vol), in 100 mM 1,4-piperazinediethanesulfonic acid (Pipes), pH 6.9/2 mM ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetate (EGTA)/1 mM $MgSO_4$/0.3 mM PMSF at 4°C. Each cortex was removed and homogenized in less than 20 sec from time of decapitation. The pooled homogenate was centrifuged at 186,000 × *g* for 60 min at 4°C. The resultant supernatant was used as the brain cytosol for all experiments. A crude tubulin preparation (T-1) that was partially depleted of calmodulin was prepared from brain cytosol by one cycle of microtubule polymerization as described (18). Tubulin marker protein was prepared by multiple cycles of microtubule assembly and disassembly (18). Tubulin from the fourth cycle of polymerization (T-4) was used in some experiments in addition to T-1 tubulin for phosphorylation studies (Table 1). Highly enriched synaptosome fractions were prepared from rat brain as described (10). Calmodulin was prepared from rat brain (13). All preparation solutions contained 0.3 mM PMSF.

Freshly prepared protein samples (80–120 μg) were analyzed for protein content and incubated under standard conditions for endogenous phosphorylation studies (12). Concentration of free Ca^{2+} in the final reaction mixture was determined with a Ca^{2+}/EGTA buffer system (19) and verified with a Ca^{2+}-specific electrode (Orion) (Fig. 5*C*) (20). The concentration of standard free Ca^{2+} was 5 μM unless otherwise specified. Final reaction volume was 0.1 ml. Tubes were incubated for 1 min at 37°C and reactions were terminated with 50 μl of $NaDodSO_4$ stop solution (12) for one-dimensional polyacrylamide gel electrophoresis or with 100 μl of a urea stop solution (solution A of ref. 21, but with twice the concentration of components) for two-dimensional polyacrylamide gel electrophoresis. Samples were subjected to one-dimensional polyacrylamide gel electrophoresis, protein staining, autoradiography, and quantitation by standard procedures (11, 12). Two-dimensional polyacrylamide gel electrophoresis was performed by a modification of the procedure of O'Farrell (21) as described by Cotman and Taylor (22).

α-Tubulin and β-tubulin were characterized by partial proteolytic digestion with *S. aureus* protease or papain as described (23).

[^{32}P]Phosphate incorporated into α- and β-tubulin was shown to be linked to protein by phosphomonoester linkages and not to lipid or nucleic acids (11, 12). Type I cyclic AMP-stimulated protein kinase inhibitor was isolated from rat brain cere-

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Abbreviations: EGTA, ethylene glycol bis(β-aminoethyl ether)-*N,N,N',N'*-tetraacetate; PMSF, phenylmethylsulfonyl fluoride.

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bellum (24). It effectively inhibited cyclic AMP protein kinases isolated from rat brain (25) and adrenal medulla (24).

RESULTS

Ca^{2+}-Activated Phosphorylation of α- and β-Tubulin. Brain cytosol was phosphorylated under standard conditions in the presence or absence of Ca^{2+}. Fig. 1 shows that the levels of phosphorylation of a doublet at 79,000–81,000 daltons, a group of bands at 51,000–64,000 and 15,000–30,000 daltons, and several minor bands were stimulated by Ca^{2+}. Tubulin is present in brain cytosol (1), and two of the major phosphoproteins visible in Fig. 1 had apparent molecular weights similar to those of α- and β-tubulin. Purified α- and β-tubulin comigrate with these two bands whose phosphorylation is stimulated by Ca^{2+} (Fig. 1). Two-dimensional polyacrylamide gel electrophoresis was used to characterize more clearly these proteins by both molecular weight and isoelectric points.

Endogenous α- and β-tubulin in brain cytosol can be clearly identified on two-dimensional gel electrophoresis by apparent molecular weight, isoelectric point, characteristic shape of protein-staining patterns, and comigration with marker tubulin (Fig. 2). Autoradiography with the two-dimensional electrophoresis system demonstrated that a major percentage of the Ca^{2+}-stimulated phosphorylation associated with α- and β-tubulin on one-dimensional electrophoresis was also associated with the staining of these proteins on two-dimensional electrophoresis (Fig. 3). The [^{32}P]phosphate incorporated into these two major bands not only comigrated with α- and β-tubulin but also had the characteristic shape of the tubulin doublet. A third independent criterion (primary structure) was used to identify α- and β-tubulin as the Ca^{2+}-stimulated phosphoproteins seen by two-dimensional electrophoresis. Partial proteolytic digests with different proteases were made of the phosphoproteins comigrating with marker α- and β-tubulin on two-dimensional gel electrophoresis, and the peptide fragments from these digests were analyzed by one-dimensional gel electrophoresis (23)

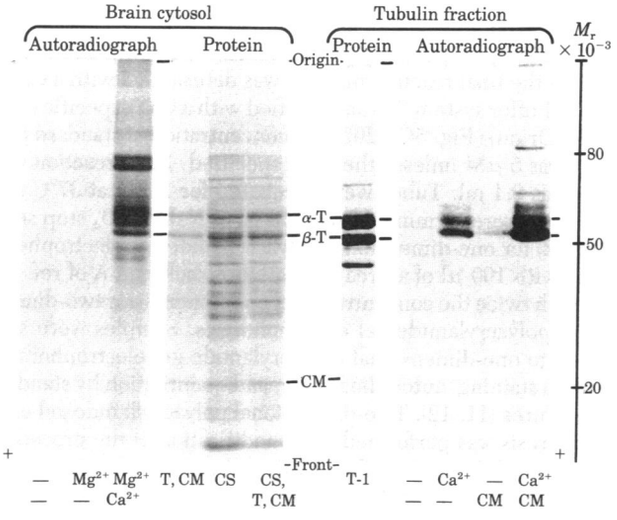

FIG. 1. Effects of Mg^{2+} and Ca^{2+} on protein phosphorylation in brain cytosol (*Left*) and of Ca^{2+} and calmodulin (CM) on protein phosphorylation in tubulin fractions (*Right*). Protein fractions were incubated under standard conditions with Mg^{2+} (4 mM), Ca^{2+} (5 μM), or calmodulin (10 μg). Protein patterns for brain cytosol (CS): tubulin (T, 10 μg) plus calmodulin (CM, 5 μg); CS (85 μg); CS (40 μg) plus T (10 μg) plus CM (5 μg). Protein pattern for tubulin: T-1 (40 μg). The protein staining band near the origin in the T-1 tubulin preparation is microtubule associated proteins. Significant Mg^{2+} stimulation of phosphorylation of these proteins is not seen under these conditions (see text).

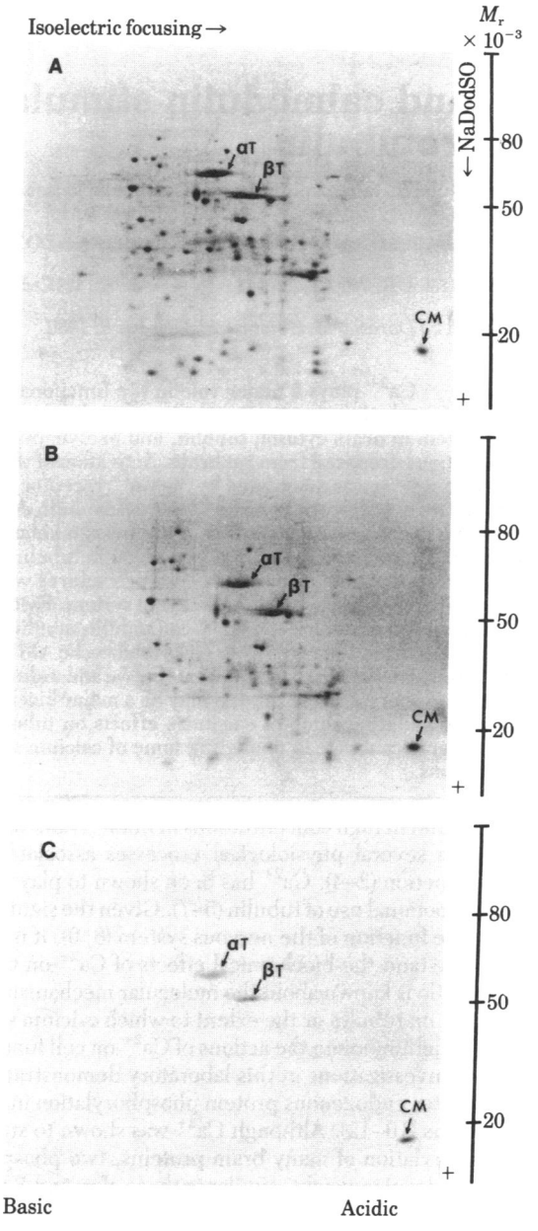

FIG. 2. Two-dimensional protein patterns of (*A*) brain cytosol (100 μg), (*B*) brain cytosol (65 μg) plus tubulin (5 μg) and calmodulin (3 μg), and (*C*) tubulin (5 μg) and calmodulin (3 μg). Positions of α- and β-tubulin (T) and calmodulin (CM) are shown.

for both protein staining and radioactivity (Fig. 4). Comparison of the *S. aureus* protease and papain digests for α- and β-tubulin with the radioactivity of these peptide maps demonstrated that most of the radioactivity associated with α- and β-tubulin was also associated with the specific proteolytic breakdown products of these proteins (Fig. 4). The sites of phosphorylation on the tubulin molecule appear to be restricted to specific regions; not all peptide fragments incorporated significant radioactivity. Peptide mapping with multiple enzymes is considered highly definitive in identifying a specific protein (23). These results plus the data obtained from one-dimensional and two-dimensional gel electrophoresis thus demonstrate that Ca^{2+} stimulates the endogenous phosphorylation of α- and β-tubulin in brain cytosol.

The concentration of free Ca^{2+} required to produce half-maximal stimulation of tubulin phosphorylation was 0.8 μM (Fig.

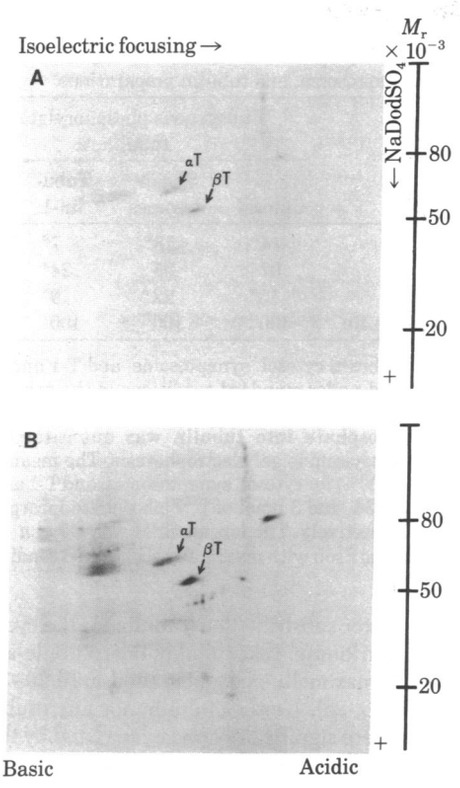

FIG. 3. Autoradiographs of two-dimensional gels of brain cytosol incubated under standard conditions in the absence (*A*) or presence (*B*) of 5 μM free Ca^{2+}. Radioactive spots comigrating with the protein-staining spots of α- and β-tubulin are shown. Their shapes are essentially identical to the staining patterns of α- and β-tubulin in the gel from which the autoradiograph was made (Fig. 2). The autoradiographs presented are representative of 10 individual experiments.

5C). The concentrations of ATP producing half-maximal and maximal phosphorylation of tubulin were 130 μM and 280 μM, respectively. Maximal [^{32}P]phosphate incorporation into protein was reached in 60 sec at 37°C. Under maximally stimulating conditions, approximately 16% of total brain tubulin was phosphorylated.

Highly enriched preparations of brain synaptosomes were incubated under standard conditions in the presence or absence of Ca^{2+} and analyzed by two-dimensional gel electrophoresis to determine if synaptosomal α- and β-tubulin were phosphorylated by a Ca^{2+}-stimulated kinase. Ca^{2+} stimulated the endogenous incorporation of [^{32}P]phosphate into α- and β-tubulin in synaptosomal preparations (Table 1). These results indicate that the Ca^{2+}-stimulated tubulin kinase system is present in preparations of synaptic nerve terminals.

Calmodulin-Stimulated Ca^{2+} Tubulin Kinase Activity. Calmodulin modulates the effects of Ca^{2+} on protein kinase activity in brain (13, 14, 26). Exogenous calmodulin did not significantly stimulate the incorporation of [^{32}P]phosphate into α- and β-tubulin when added to brain cytosol (Table 1). This result is not surprising because calmodulin is present in high concentrations in brain cytosol (27), indicating that this fraction is already saturated with the Ca^{2+} receptor protein (Fig. 2). To demonstrate an effect of calmodulin on the Ca^{2+} tubulin kinase system, it was necessary to obtain a calmodulin-depleted preparation that also contained both tubulin and the tubulin kinase system. To prepare such a fraction, we isolated tubulin from brain cytosol by one cycle of microtubule polymerization. Tubulin represented ≈55% of the total protein in this T-1 fraction; the majority of calmodulin in brain cytosol remained in the supernatant fraction, leaving only a small fraction of calmodulin in the T-1 tubulin preparation (Fig. 1).

Calmodulin caused a marked stimulation of Ca^{2+}-dependent tubulin kinase activity when added to the T-1 fraction (Fig. 1 and Table 1). The concentrations of calmodulin required to produce a half-maximal increase in Ca^{2+}-stimulated phosphorylation of α- and β-tubulin were 0.35 and 0.45 μg, respectively (Fig. 5*D*). Calmodulin alone had no significant effect on tubulin kinase activity (Table 1). Tubulin isolated by four cycles of microtubule assembly and disassembly (T-4) was essentially free of calmodulin and had no Ca^{2+}-calmodulin tubulin kinase activity (Table 1). These results reveal that both calmodulin and the tubulin kinase systems were removed from tubulin during multiple cycles of microtubule assembly, strongly suggesting that this

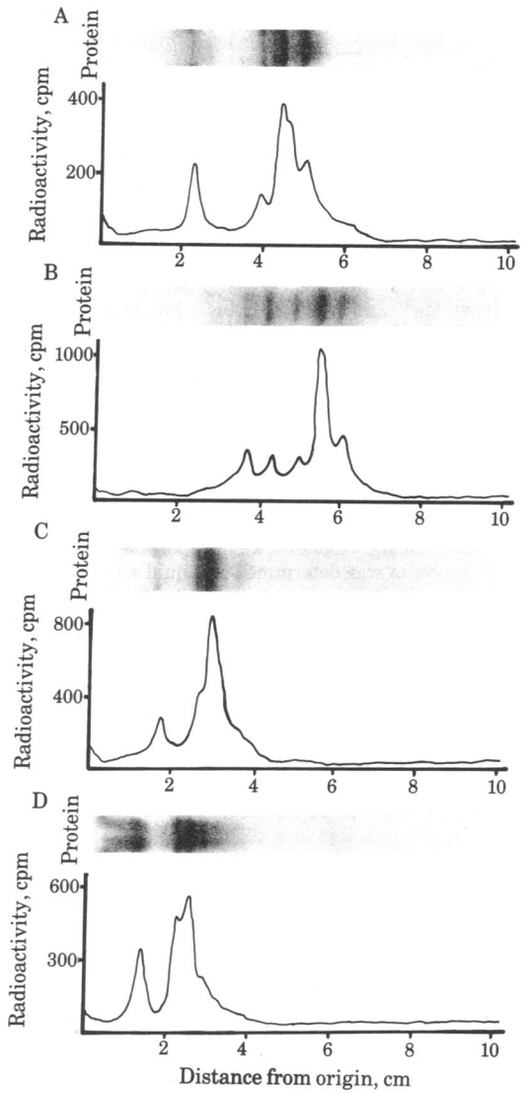

FIG. 4. Peptide maps and associated radioactivity of α- and β-tubulin from brain cytosol. Brain cytosol (30 μg) was phosphorylated under standard conditions in the presence of Ca^{2+}, mixed with 40 μg of marker tubulin, and subjected to two-dimensional polyacrylamide gel electrophoresis (Figs. 2 and 3). Protein-staining bands corresponding to marker α- and β-tubulin were cut from the gel and digested with *S. aureus* protease or papain. Peptides were mapped on one-dimensional gels as described (23). Incorporation of [^{32}P]phosphate into protein bands along the peptide map is expressed in cpm (12). (*A*) α-Tubulin plus *S. aureus* protease; (*B*) β-tubulin plus *S. aureus* protease; (*C*) α-tubulin plus papain; (*D*) β-tubulin plus papain.

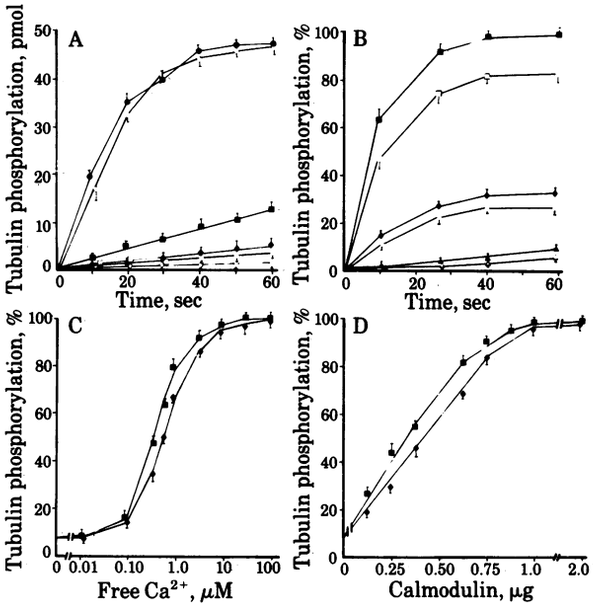

FIG. 5. (*A*) Effects of cyclic AMP (type I) protein kinase inhibitor on Ca^{2+}- and cyclic AMP-stimulated tubulin phosphorylation. Brain cytoplasm was incubated under standard conditions in the presence of Ca^{2+} and Mg^{2+} (●), Ca^{2+} and Mg^{2+} plus inhibitor protein (○), Mg^{2+} and cyclic AMP (■), Mg^{2+} and cylic AMP plus inhibitor protein (□), Mg^{2+} alone (◆), and Mg^{2+} plus inhibitor protein (◇). Concentrations of Mg^{2+}, free Ca^{2+}, cyclic AMP, and inhibitor protein in the reaction tubes were 4 mM, 5 μM, 10 μM, and 10 μg, respectively. Data are expressed as pmol of [^{32}P]phosphate incorporated into tubulin per mg of cytosol protein ($n = 7$; error bars, mean ± SEM). (*B*) Time course of Ca^{2+}- and calmodulin-stimulated [^{32}P]phosphate incorporation into α-tubulin (open symbols) and β-tubulin (solid symbols). T-1 tubulin was incubated under standard conditions for various times in the presence of 5 μM free Ca^{2+}, 4 mM Mg^{2+}, and 10 μg of calmodulin (□, ■), 5 μM Ca^{2+} and 4 mM Mg^{2+} (◇, ◆), or 4 mM Mg^{2+} alone (△, ▲). Incorporation of [^{32}P]phosphate into α- and β-tubulin was determined by liquid scintillation spectrometry of the tubulin-staining bands (18) ($n = 10$). (*C*) Effects of Ca^{2+} concentration on tubulin phosphorylation in brain cytosol (◆) and T-1 tubulin (■) preparations ($n = 20$). Each protein fraction was incubated under standard conditions (Fig. 1) at pH 7.1, and the concentration of free Ca^{2+} was varied by using the Ca^{2+}/EGTA buffer (0.4 mM EGTA). (*D*) Activation of the tubulin kinase system by calmodulin in T-1 tubulin preparations. Protein was incubated under standard conditions in the presence of Ca^{2+} and in the presence or absence of calmodulin. Incorporation of [^{32}P]phosphate into α-tubulin (■) and β-tubulin (◆) was determined as described for Fig. 4*B* ($n = 15$).

enzyme system is not required for initiation of microtubule polymerization.

Comparison of Ca^{2+}- and Cyclic AMP-Stimulated Tubulin Kinase Systems. Cyclic AMP-stimulated protein kinase activity copurifies with tubulin (15, 16, 28). To determine whether the Ca^{2+}-calmodulin tubulin kinase is distinct from the cyclic nucleotide-Mg^{2+} tubulin kinase, a selective protein inhibitor of cyclic AMP-dependent protein kinase (type I inhibitor) (24) was isolated from brain to determine its effects on these tubulin kinases (Fig. 5*A* and Table 2). Type I protein kinase inhibitor had no significant effect on Ca^{2+}-stimulated tubulin kinase activity in brain cytosol (Fig. 5*A*) or T-1 tubulin (Table 2) preparations but did inhibit Mg^{2+}- and cyclic AMP-stimulated tubulin kinase activity in these preparations. Ca^{2+} also caused maximal stimulation of [^{32}P]phosphate incorporation into tubulin in less than 1 min, whereas the cyclic AMP tubulin kinase system required 20 min or longer to reach maximal levels of phosphorylation (15, 16, 28) (Fig. 5*A*).

Jameson *et al.* (28) have shown that microtubule-associated proteins are a better substrate than tubulin for the cyclic AMP-stimulated tubulin kinase. Like tubulin, microtubule-associated proteins are not maximally phosphorylated until 20–30 min of incubation (15, 16, 28). Neither tubulin nor microtubule-associated proteins were significantly phosphorylated by this kinase under the standard 30-sec incubation conditions, which produce nearly maximal activation of the Ca^{2+}-calmodulin tubulin kinase (Fig. 1). At much longer incubation times, the activity of the cyclic AMP-stimulated kinase became more apparent (when inhibitor was left out of the incubation mixture) (Fig. 5 *A* and *B*). Ca^{2+} and calmodulin did produce some stimulation of the endogenous phosphorylation of microtubule-associated proteins, but unlike the cyclic AMP kinase system, the phosphorylation of microtubule-associated proteins by the Ca^{2+} kinase was minor compared to tubulin (Fig. 1).

The Ca^{2+}-calmodulin tubulin kinase was removed from tubulin after two cycles of microtubule assembly–disassembly,

Table 1. Effects of Ca^{2+} and calmodulin on endogenous phosphorylation of tubulin in brain cytosol, synaptosome, and tubulin preparations

Condition	Endogenous phosphorylation of tubulin, %			
	Cytosol	Synaptosomes	Tubulin-1	Tubulin-4
Control	14*	18*	7*	100
Ca^{2+}	97	95	24*	82*
Calmodulin	16*	22*	9*	98
Ca^{2+} and calmodulin	100	100	100	79*

Preparations of brain cytosol, synaptosome, and T-1 and T-4 tubulins were incubated under standard conditions in the presence or absence of Ca^{2+} (5 μM) or calmodulin (10 μg) or both, and the incorporation of [^{32}P]phosphate into tubulin was quantitated by two-dimensional polyacrylamide gel electrophoresis. The means of maximal stimulation (100%) for cytosol, synaptosomes, and T-1 and T-4 tubulins were 50, 21, 34, and 3 pmol of [^{32}P]phosphate incorporated per mg of protein, respectively. The largest SEM was ±7%; $n = 4$.
* $P < 0.001$ in comparison with maximally stimulated conditions (Student's *t* test).

Table 2. Effects of type I kinase inhibitor, prolonged preparation time, and absence of PMSF in preparation media on endogenous activity of Ca^{2+}-calmodulin and cyclic AMP tubulin kinase systems in tubulin-1 preparations

Condition	Endogenous phosphorylation of tubulin, %	
	Ca^{2+}-calmodulin tubulin kinase activity	Cyclic AMP tubulin kinase activity
Control	100	100
Type I kinase inhibitor	99.2 ± 1.1	24.1 ± 3.2*
Prolonged preparation	11.3 ± 2.1*	92.4 ± 3.2
Absence of PMSF	24.2 ± 3.6*	98.3 ± 4.1

T-1 tubulin preparations were prepared under control (standard) and various conditions and incubated in the presence of Ca^{2+} and calmodulin (Ca^{2+}-calmodulin tubulin kinase activity) or cyclic AMP (cyclic AMP tubulin kinase activity) with or without type I protein kinase inhibitor. Incorporation of [^{32}P]phosphate was determined as described in the legend of Table 1. Data give the means ± SEM; $n = 10$. The means of maximal stimulation (100%) for Ca^{2+}-calmodulin and cyclic AMP tubulin kinase activity were 40 and 7 pmol of [^{32}P]phosphate per mg of protein, respectively. Control conditions represent T-1 tubulin isolated by standard procedures. For prolonged preparation conditions, rat brains were removed 7–8 min instead of 20 sec after decapitation. For absence of PMSF condition, PMSF was omitted from all media during preparation of T-1.
* $P < 0.001$ in comparison with maximally stimulated conditions.

whereas cyclic AMP-Mg^{2+} kinase activity was still present in tubulin prepared by four cycles, T-4 (Table 1). Ca^{2+} also inhibited the activity of the cyclic AMP tubulin kinase in the more purified tubulin fractions (Table 2), as described for several other cyclic AMP protein kinase systems. The stabilities of these two kinase systems were also different. Cyclic AMP tubulin kinase activity was not significantly affected if PMSF was omitted from the homogenization buffer or if brains were removed up to 7–8 min after decapitation (Table 2). However, Ca^{2+}-calmodulin tubulin kinase activity was essentially lost if brains were not rapidly homogenized or if PMSF was omitted from the preparation (Table 2). These results strongly indicate that Ca^{2+}-calmodulin tubulin kinase is distinct from the previously characterized cyclic AMP-dependent tubulin kinase (15, 16).

DISCUSSION

Our results elucidate a novel biochemical effect of Ca^{2+} on tubulin. Ca^{2+} significantly stimulated the endogenous phosphorylation of tubulin in brain cytosol and synaptosome preparations. The effects of Ca^{2+} on the level of endogenous tubulin phosphorylation are modulated by calmodulin. The evidence indicates that the Ca^{2+}-calmodulin tubulin kinase system is distinct from the previously described cyclic AMP-Mg^{2+} tubulin kinase (15, 16, 28, 29). The fact that tubulin, a major structural component of cells, is also a major substrate for Ca^{2+}- and calmodulin-dependent kinase activity in brain indicates that this system may be important in the control of some aspects of cellular and neuronal function and in mediating some of calcium's effects on physiological processes.

The numerous functions of tubulin (2–4) and the role of Ca^{2+} in modulating tubulin-containing systems in brain (5–7) have been well documented. Because highly purified tubulin that shows no Ca^{2+}-stimulated endogenous phosphorylation can still polymerize into microtubules (Table 1), it is unlikely that endogenous Ca^{2+}- and calmodulin-stimulated phosphorylation of tubulin is directly involved in microtubule assembly, but it may be indirectly involved by regulating the availability of tubulin for the assembly process or by mediating other roles of tubulin in cell function. Phosphotubulin may be a structural element in unidentified cellular components. Recent studies in this laboratory show that tubulin, calmodulin, and the tubulin kinase are associated as a complex in cytosol. Phosphorylation of tubulin by this Ca^{2+}-calmodulin kinase system causes marked changes in the physiochemical properties of tubulin, resulting in the temperature-dependent formation of nonrandom, insoluble, "filamentous-like" structures clearly distinct from microtubules when viewed by electron microscopy. The role of these Ca^{2+}- and calmodulin-regulated tubulin structures in cell function is not known.

Tubulin is found in high concentrations in preparations of whole nerve terminals (25, 30) and synaptosomal subfractions, including synaptic junctional membrane (22) and vesicles (31). However, microtubules are rarely seen in nerve terminals (30–33), raising the question as to the function(s) of synaptic tubulin. Our results show that phosphorylation of tubulin in synaptic preparations is dependent upon Ca^{2+} and calmodulin (Table 1). Further, results (to be published elsewhere) establish that membrane-bound tubulin in synaptic vesicles is phosphorylated by an endogenous Ca^{2+}-calmodulin-dependent kinase system. The suggested roles for tubulin (30, 34), calmodulin (10, 35), and Ca^{2+}-dependent protein phosphorylation (13, 14) in neurotransmitter release and synaptic modulation led us to hypothesize that the Ca^{2+}- and calmodulin-dependent phosphorylation of tubulin in synaptosome preparations may mediate some of calcium's effects on synaptic function, such as transmitter release or vesicle membrane interactions.

Although the physiological significance of the Ca^{2+}- and calmodulin-dependent phosphorylation of tubulin remains to be fully elucidated, the ability of tubulin to serve as an endogenous substrate for Ca^{2+}-calmodulin tubulin kinase systems represents an important new property of tubulin. The calmodulin kinase may serve to modulate the effects of Ca^{2+} on the state of aggregation or the functional properties of cytoplasmic and membrane-bound forms of tubulin proteins.

We thank Drs. G. Glaser, G. Palade, and J. Pincus for critical readings of the manuscript and Drs. R. Yu, A. Kleinhaus, F. Richards, and T. Seyfried for useful discussions. This work was supported by Research Career Development Award NSI-EA-1-K04-NS 245 and U.S. Public Health Service Grant NS 13532 to R.J.D.

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